Metallic nanoparticles as synthetic building blocks for cancer nanodiagnostics: from materials design to molecular imaging applications

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Metallic nanoparticles have been a matter of intense exploration within the last decade due to their potential to change the face of the medical world through their role as 'nanotheranostics'. Their envisaged capacity to act as synthetic platforms for a multimodal imaging approach to diagnosis and treatment of degenerative diseases, including cancer, remains a matter of lively debate. Certain synthetic metal-based nanomaterials, e.g. gold and iron oxide nanoparticles, are already in clinical use or under preclinical investigations following their *in vitro and in vivo* preclinical imaging success. We surveyed the recent publications landscape including those reported metallic nanoparticles which are already having established applications *in vivo*, as well as some of the new metallic nanoparticles which, despite their potential as cancer nanodiagnostics are currently awaiting *in vitro* and *in vivo* evaluation. The objective of this review is to highlight the current metallic nanoparticles, alloys and their derivatives with multimodal imaging potential with a main focus on their chemistry as a springboard to discussing their role in the future of nanomedicines design. We highlight here some of the fundamentals of molecular and nano-imaging techniques of relevance to metal-based colloids, alloys and metallic nanoparticles and their future prospects as cancer nanodiagnostics. The current approaches for metallic and alloy surface derivatisation, aiming to achieve functional and biocompatible materials for multimodal bioimaging applications, are discussed aiming to address some new perspectives on the efficiency of metallic nanoparticles as synthetic scaffolds for imaging probe design and their use in medical imaging techniques (CT, PET, SPECT and MRI).

1. Introduction and current importance of the field

The National Institutes of Health currently defines nanomedicine as a wide range of nanoscale technologies aimed to revolutionise the bases of disease diagnosis, treatment, and prevention.[1] Nanomedicine is concerned with the medical application of nanotechnology, with a focus ranging from nanomaterials design to nanobiosensors and their possible future applications to molecular scale technology.[2,3] This field is currently much hyped in the press due to its potential to address challenges in imaging and treatment of degenerative diseases, including cancer.

One of the most overwhelming reasons for the increased research attention in drug discovery[4-7] towards nanomedicines is due to the fact that cancer is one of the top ten leading causes of death in the world. According to a cancer statistics report from World Health Organization (WHO) there were 14.1 million adults diagnosed with cancer and 8.2 million (58.16%) deaths from cancer in the world in 2012.[8] The Surveillance, Epidemiology, and End Results (SEER) Program works to provide information on cancer statistics using data from a wide range of geographical locations. The SEER Program reviewed that the earlier the stage at which the tumour was diagnosed, the higher the survival rate would be. For example, until very recently, only 15% of lung cancer cases were diagnosed at the localised stage, and 54% of such patients could survive. 22% of cancer incidence was detected at the spreading stage (adjacent), which resulted in a reduced survival rate of 26% (Figure 1). This survey highlights that the majority of patients were diagnosed at a late stage, hence only 4% could survive five years post diagnosis. Thus, it could be concluded that early cancer diagnosis is equally important as cancer treatment, an early and precise diagnosis of cancer could significantly improve patients' survival rate or enable the possibly of successful treatment *via* surgical removal. At present, there are four methods which are used in clinical practise to diagnose cancer, i.e. medical imaging,[9] endoscopy,[10] biopsy[11] and blood or other sample testing.[12,13] Blood sample tests are also used to reveal cancer by targeting overexpressed molecules, including sugars, fats, proteins, RNA and DNA. However, there remains selectivity or sensitivity problems in the current testing reagents.[14] Biopsy continues to be the most certain way to diagnose cancer; whereby a tissue sample is collected from the site of interest. Subsequently the morphology and gene status is examined.

However, it is still the case that it is usually difficult to locate a site of a tumour. Therefore, molecular imaging has the potential to speed up the diagnosis in combination with blood sample testing to allow location of a tumour or early cancer detection,[15] including more recently near IR imaging investigations using advanced endoscopy for colorectal cancer and breast imaging. Other methods, more commonly used in the diagnosis of tumours which are difficult to access, involve optical fluorescence imaging. These involve the use of fluorophores and, for enhanced imaging, quantum dots (QDs) are recently used as part of endoscopes.

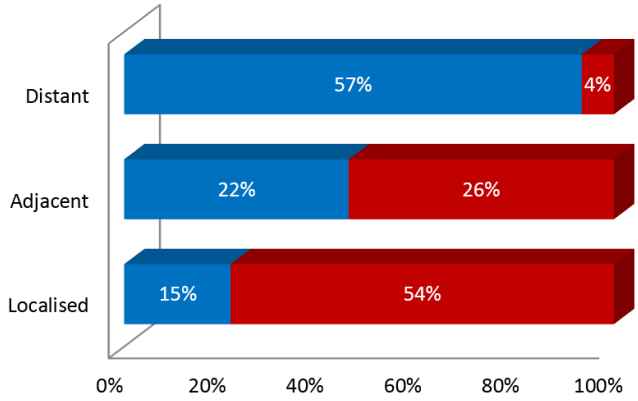

Figure 1. Lung cancer diagnosis and survival by stage, 2003-2009. Blue: Stage at which the cancer was diagnosed; Red: 5-year survival rate.

In general, endoscopy is applied at the middle or late stages of cancer diagnosis and is combined with other imaging tools to confirm a cancer diagnosis or with biopsy to collect a tissue sample for further investigations.[15] Medical imaging techniques currently used in cancer diagnosis include X-ray imaging,[16] X-ray computed tomography (CT scans),[16,17] Magnetic Resonance Imaging (MRI)[18] and Positron Emission Tomography (PET). Much like PET, single photon emission computed tomography (SPECT) uses a similar principle to detect pathologies; however, due to its reliance on a single gamma ray emitted by the radioactive probe, the degree of sensitivity is a factor of 100 lower than that of PET.[17] These techniques use one source of energy (X-ray, magnetism, gamma or positron decay) to create a detailed view of the body to locate a tumour mass. Medical imaging techniques constitute a matured field, and these techniques are used to detect all types of cancer.

Most medical imaging methods rely heavily on the use of radioisotopes as contrast agents. For example, positron emission tomography (PET) is a nuclear imaging method that uses positron (β^+)-emitting radioisotopes such as those of oxygen (^{14}O $t_{1/2}$= 1.2 min,^{15}O $t_{1/2}$= 2.1 min), nitrogen (^{13}N $t_{1/2}$= 10.0 min), carbon (^{11}C $t_{1/2}$= 20.3 min), and fluorine (^{18}F $t_{1/2}$= 109.7 min), but also some unconventional metallic radioisotopes of Cu (^{61}Cu $t_{1/2}$= 3.35 h, ^{62}Cu $t_{1/2}$= 4.7 min, ^{64}Cu $t_{1/2}$= 12.7 h, ^{64}Cu $t_{1/2}$= 61.9 h), Zn (^{62}Zn $t_{1/2}$= 9.2 h, ^{65}Zn $t_{1/2}$= 243.7 d), ^{38}K ($t_{1/2}$= 7.6 min), ^{82}Rb ($t_{1/2}$= 1.3 min), ^{32}P ($t_{1/2}$= 14.3 d), ^{59}Fe ($t_{1/2}$= 44.5 d), Ga (^{64}Ga $t_{1/2}$= 2.6 min, ^{67}Ga $t_{1/2}$= 78.3 min, ^{68}Ga $t_{1/2}$= 68.1 min) and the halogen series are entering the molecular imaging arena with some at the clinical research stage. In PET two gamma rays signals are detected (occurring at 180 degree angle), which are produced by a positron annihilation event at the target, with a discernible image subsequently produced and interpreted computationally. Other metallic isotopes that are of interest to multimodal early diagnosis and treatment approaches include ^{90}Y ($t_{1/2}$= 64 h) and ^{86}Y ($t_{1/2}$= 14.7 h). ^{68}Gallium is also becoming a popular radioisotope in clinical research as it is available from generators, and research showed that, when anchored onto a suitable *in vivo* delivering vehicle in precancerous stages, it selectively concentrates around infected sites, areas of inflammation, and can report on areas of rapid cell division. The longer lived metallic isotopes are thought to become more promising in future diagnostic imaging applications than ^{68}Ga, since their longer circulation *in vivo* allows for improved tumour/background ratio enhancement.[19]

The other focus of much current research is the problem of how to deliver diagnostic or therapeutic reagents in low molecular doses extremely precisely to a desired location in the body. The design of a successful drug delivery system is determined by many factors, and it is believed that design of nanomedicines can play a crucial role here in the future. As vectors for drug delivery, nanoparticles offer a range of unique advantages including their ability to modify drug pharmacokinetics *in vivo* and their capacity to be loaded with high concentrations of different drugs for advancing combination therapies and theranostics.[20-25] Nanoparticle properties can be tailored to control drug release, modify blood circulation half-lives, improve biodistribution profiles, increase tissue permeability and target specificity, as well as enhanced metabolic stability.[26]

This includes the choice of types of vehicle used for drug delivery, size and shape, methods of functionalisation, ease of incorporation of the contrast agent (radioactive or not), issues of cellular and tumour microenvironment uptake as well as general side effects. The nature of the nano-vehicle chosen and a robust design may in fact be the first and most important step, which would directly as well as indirectly affect the subsequent steps in design, synthesis and imaging *in vivo* for a diagnostic nanomedicine. Currently, there are nine different nanocarrier types which are most widely used in biomedical research and have applications with relevance to tumour diagnosis and treatment (Figure 2).

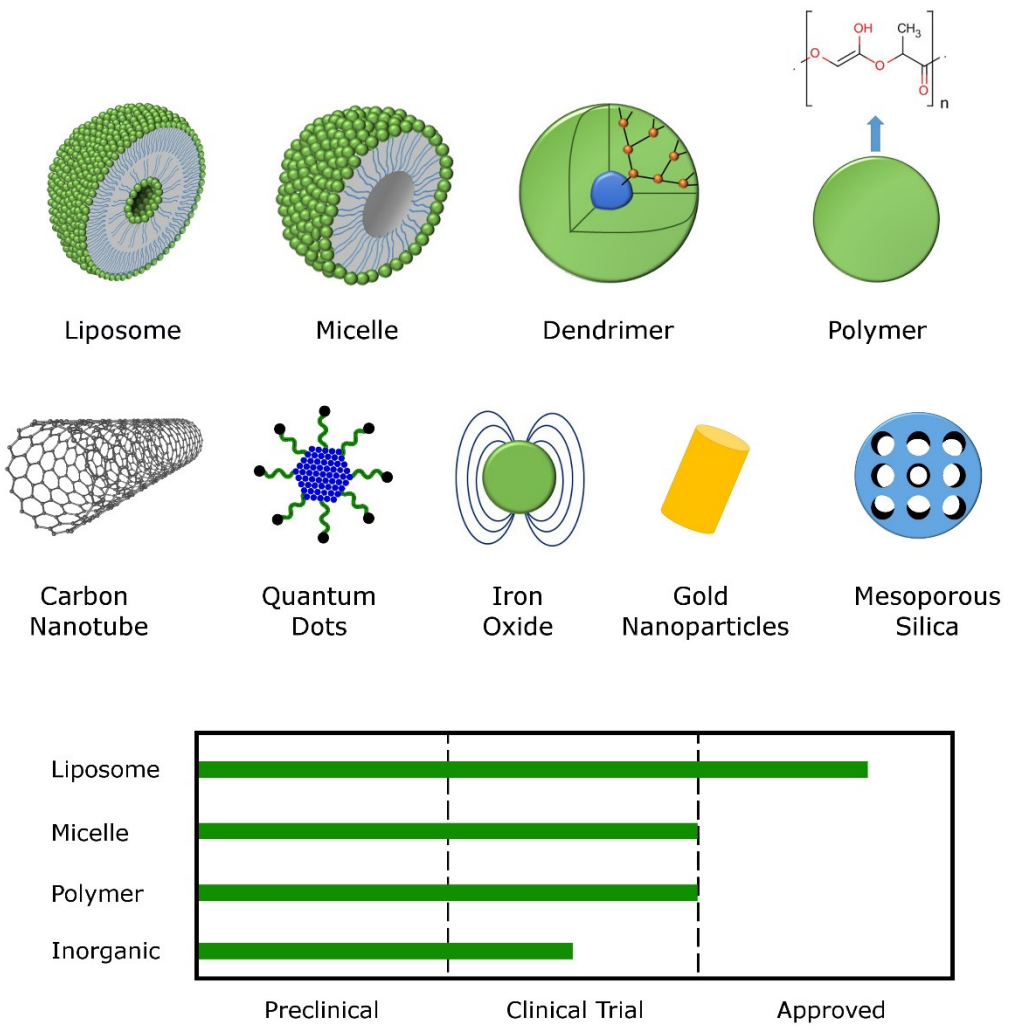

Figure 2. General categories of nanocarrier scaffolds and their current development stages

As shown in Figure 2, most of the nanocarriers of current clinical or pre-clinical research interest can be largely categorised into four groups: lipid-based, surfactant-based, polymer based and inorganic based systems. Liposomes discovered in 1965, constituted the first nanovehicles which entered the nanomedicine and drug delivery research arena, where phospholipids were one of the few solubilising systems which could be well tolerated *in vivo* with the capacity to increase the solubility of the encapsulated drug a hundred to ten thousand fold. Followed by nearly 50 years of intensive research, the application of liposomes in biomedicine was approved for clinical use. Both micelle and polymeric vesicles were less popular than the lipid-based system, hence, the development of these two types of carriers advanced slower than that of liposomes.

In recent years, inorganic nanocarriers have become more and more popular due to their sizes, shapes and ability to act as nanomedicines scaffolds, as well as their intrinsic physicochemical unique properties, e.g. magnetism, emission/absorption spectroscopies, etc. Most inorganic carriers are currently at a preclinical and clinical trial stage. The most common of these, quantum dots, are intensely fluorescent and it was already demonstrated that these may be further bound to anticancer reagents to make the resulting dual imaging and therapeutic composition traceable in the tissue (discussed elsewhere in this themed issue). At the other end of the spectrum in terms of proximity to clinical use, single-walled carbon nanotubes (SWNTs) have a rigid framework and can absorb near infrared (NIR) light to generate heat, which could be used for photo thermal therapy of cancer treatment. There have been several other reviews with a focus on the development of single-walled nanotubes and their biocompatibility, which raise awareness of their inherent toxicity.[27,28]

The general overview of metallic nanoparticles with a focus on their preclinical development stage for cancer diagnosis has not been tackled in recent years and is therefore reviewed hereby. Furthermore, iron oxides are probably the most popular nanomaterials in clinical use: they are magnetic, can be rapidly derivatised and combined with fluorescent probes to generate a dual modality imaging and/or sensing complex or nanohybrid. For these, a core shell design in nanotheranostics (a nanoscale amalgamation of diagnostics and therapeutics) is a relatively new but promising field with multidirectional applications, potentially revolutionising the efficacy of diagnostic nanomedicines and in the long-term their use as combined therapeutics, for patient benefit. This offers a targeted approach on a cellular level with future applications on a molecular scale which still currently represents an unmet clinical need for improved diagnostic contrast agent design. Robert A. Freitas first used the term ‘medical nanotechnology’ in a report in 1998. Since then, multiple nanomedicine-dedicated journals have begun, and funding from investors is by no means a limiting factor. In the last decade the “nanomedicine” term has gone from being a term only

used in science fiction, to a practical reality in the design of medicines and opened up new clinical and pre-clinical research areas.

Small molecule-based, contrast agents afford *in vivo* diagnostics, greatly increasing the sensitivity of otherwise undetectable pathologies.[29] Nano-medical advances further increase this diagnostic potential by opening up new possibilities of imaging technologies and applications, such as fluorescence imaging. There are many different types of nanomaterial currently under development – with some even at the clinical phase, such as gadolinium chelates and salts (Magnevist®), and iron oxide nanoparticles.[30] Nanomedicine offers use of current imaging technologies, including new opportunities in fluorescent tagging, which until now, has not been widely used due to poor signal strength, wide emission bands *in vivo*, and poor tissue penetration.[29] It offers even more insight into disease progression and can give information on tumour uptake mechanisms.[31] This level of depth is required in order to probe diseased tissue on a molecular level, leading to a full understanding of the mechanisms involved, and hence longer term, more effective patient diagnosis of treatment.

Here we will discuss the molecular imaging aspects and the nanodiagnostic potential with respect to simple small molecule contrast agents. This review also tackles their role as synthetic scaffolds for all-in-one integral mode, synthesis, and cytotoxicity as a means to destroy specific cancerous cells.

The communities to which this article may appeal are likely to be those with a focus on academic impact in the short term, such as physical chemists, metals in medicine and nanotechnology researchers, since these fields all play a significant role in the development of future diagnostics and therapeutics and in the emergence of this new area of molecular imaging. The rational design of novel medicines and drug delivery devices will become, in the long-term a step change in simultaneous diagnosis and treatment of tumour. It will be of international interest to chemists researching fundamental metal chemistry and nanomedicine and those developing use of metals in medicine, new therapeutics and contrast imaging agents.

2. Metallic nanoparticles-based imaging probes – challenges and potential for their use in tumour diagnosis

The application of metal complexes in microscopy, sensing and analysis is well established. While fluorescent probes offer high sensitivity, natural biological molecules absorb light effectively and many of them are also emissive. This gives rise to two problems (a) absorption and scattering of UV and Vis light by the sample limits the depth of sample that can be imaged (b) emission of light by the sample (autofluorescence) limits probe signal : background ratios. Since biological samples absorb less strongly with increasing wavelength, excitation with red light and multiphoton imaging by near infra-red (NIR) light may be used to reduce the magnitude of these problems.

Metal species with long luminescence lifetimes can be used in time-gating experiments that effectively reject scatter and short-lived fluorescence from the sample. One of the main limitations for *in vivo* optical imaging is absorption by haem in blood which blocks out the 450-550 nm region: it is essential to shift to NIR to avoid this, in addition to taking advantage of the enhanced tissue penetration in the NIR. The development of both time-gating methodologies (fluorescence lifetime microscopy) and in the application of novel probes for long wavelength using two-photon excitation is highly,[32] due to time gating, which provides a method to observe a molecular species by a change in its excited state. So, there is a high interest in the design and testing of new molecular probes based on intrinsically near IR fluorescent nanoparticulate motifs for multimodal imaging oncological applications.[33-37] In this sense, it is of particular interest to investigate *in vitro* and *in vivo* core-shell nanophosphors doped with rare earths and near infra-red emitting nanoparticles (NIR NPs), coated with a silica layer and functionalised with peptides and antibodies to target tumour delivery. The NPs encapsulation allows multiple luminescent/fluorescent ions/molecules including radioactive ions to reach the target simultaneously and prevents the loss of these imaging agents to non-specific sites, while keeping the doses of the overall nanoprobe necessary to get an image *in vitro*/*in vivo* extremely low. These NPs can be applied to the medical diagnostics market by overcoming disadvantages of existing fluorophores e.g. quantum dots (cost and toxicity) and small molecule fluorophores (short life-time < 5 ns, photobleaching, cytotoxicity). Furthermore, the long-lived emissive nanophosphors for optical imaging in the near infra-red can be tailored into nanoparticles which are simultaneously radioactive (i.e. incorporating metallic radioisotopes suitable for tracing in tissues and organs both *in vitro* and *in vivo*).

The stability of metal complexes in a biological environment must be considered when designing a new nanomaterial intended for clinical applications in cancer diagnosis. The materials should exhibit high kinetic and thermodynamic stability in order to prevent premature decomposition within true living systems.[38] Additionally, a toxic imaging agent (e.g. gadolinium(III)) must be shielded from the biological environment: a way of achieving this is by coating the metal ion in polymer layer. Moreover, chemotherapeutic agents can be attached to the coating and/or metal, crucially treating only cancerous cells, with specific volumes avoiding the toxic side effects of standard chemotherapy used in clinical practise.

In the last decades, Magnetic Resonance Imaging (MRI) has arisen as one of the most promising reported imaging methods which is increasingly making use of nanomedicines as contrast agents, and, as result, has the potential for highly accurate imaging.[39] The sensitivity of MRI can be greatly enhanced by the selective accumulation of magnetic nanoparticles at diseased sites. This may be achieved by incorporating molecules with an affinity for specific protein signatures associated with malignant cells. This relies on selective targeting of the probe. Targeting here refers to the ability of a metal-based nanoparticle to identify the target tissue and selectively accumulate within the cells. This means that the site of interest (i.e cancerous tissues) can be better differentiated from the surrounding healthy tissue, by increasing the contrast ratio.[29] Furthermore, it has been demonstrated that the use of MNPs leads to an increased signal-to-noising ratio, resulting in a higher resolution image. A significant advantage is that a lower dose of the toxic contrast agent will be present in the body; which constitutes a clear advantage to the patient, since *contrast media* are known to cause adverse reactions, and toxicity is directly proportional to the dose.

For magnetic resonance imaging, superparamagnetic nanoparticles have extended susceptibility-based contrast agents toward targeted imaging. In this sense, the strong magnetic moment in gadolinium(III) ions (i.e. paramagnetism due to

unpaired electrons) is exploited. Gd(III) as a component of contrast agents used clinically provide high contrast ratio on T_1-weighted images (an image with greater signal intensity from fat-containing tissues).[40] High stability is crucial for Gd-ligand complexes due to the associated toxicity of free Gd(III) (0.3–0.5 mmol kg^{-1}) *in vivo*. Free Gadolinium ions have a high affinity for various serum proteins and skeletal tissue, (due to the presence of Ca(II)) so it is essential that the ions stay intact prior to clearance.[41] However, chelate ligands such as DTPA (diethylenetriamine pentaacetic acid – used as a clinical agent under the trade name, Magnevist®) can be introduced onto the surface shielding the toxic Gd from the biological environment.[29] DTPA is an aminocarboxylate derivative conisisting of a diethylenetriamine backbone and five carboxymethyl groups. The ligand is chemically similar to EDTA – a common clinical chelate ligand, used in the sequestering of harmful substances. Whilst Gd–DTPA and Gd–DOTA were the first contrast agents used in a clinical context, neutral complexes such as Gd–DTPA–BMA and Gd–HPDO3A are also commonly used. Likewise, Teslascan® (mangafodipir trisodium, formerly known as Mn–DPDP) has recently been approved for liver imaging, which has attracted attention due to high paramagnetism of divalent manganese.[42] These derivatives have been summarised in Figure 3.

Ligands can be modified to optimise certain relaxation parameters *in vivo*. The factors affecting the water exchange rate of DTPA derivatives have been studied and the significance of sterics in enhancing relaxation rates has been highlighted. However, upon increasing the various carbon chain lengths of the ligand, a decrease in terms of thermodynamic stability occurs. Similar studies have been conducted on Gd-DOTA derivatives and again, positive results point to steric crowding of the Gadolinium ion. Oxygen-based donors such as hydroxypyridinone (HOPO), terephthalamide (TAM), and maltol (MAM) have been investigated as chelating agents expected to stabilise Gd complexes. The feasibility of these oxygen donor agents *in vivo* has been proven successful, and some reports show that HOPO-based chelates show even higher stability than the benchmark compounds, DTPA and DOTA.[41] An example of non-amincarboxylate co-ordinated Gd(III) contrast agents is an encapsulation of the ion in fullerene cages. The nano-dimensional carbon cages are decorated with hydrophilic groups to ensure solubilisation in the aqueous environment. However, these materials have shown relatively high toxicity in the presence of salt, limiting their practical use as contrast agents in clinical practise.

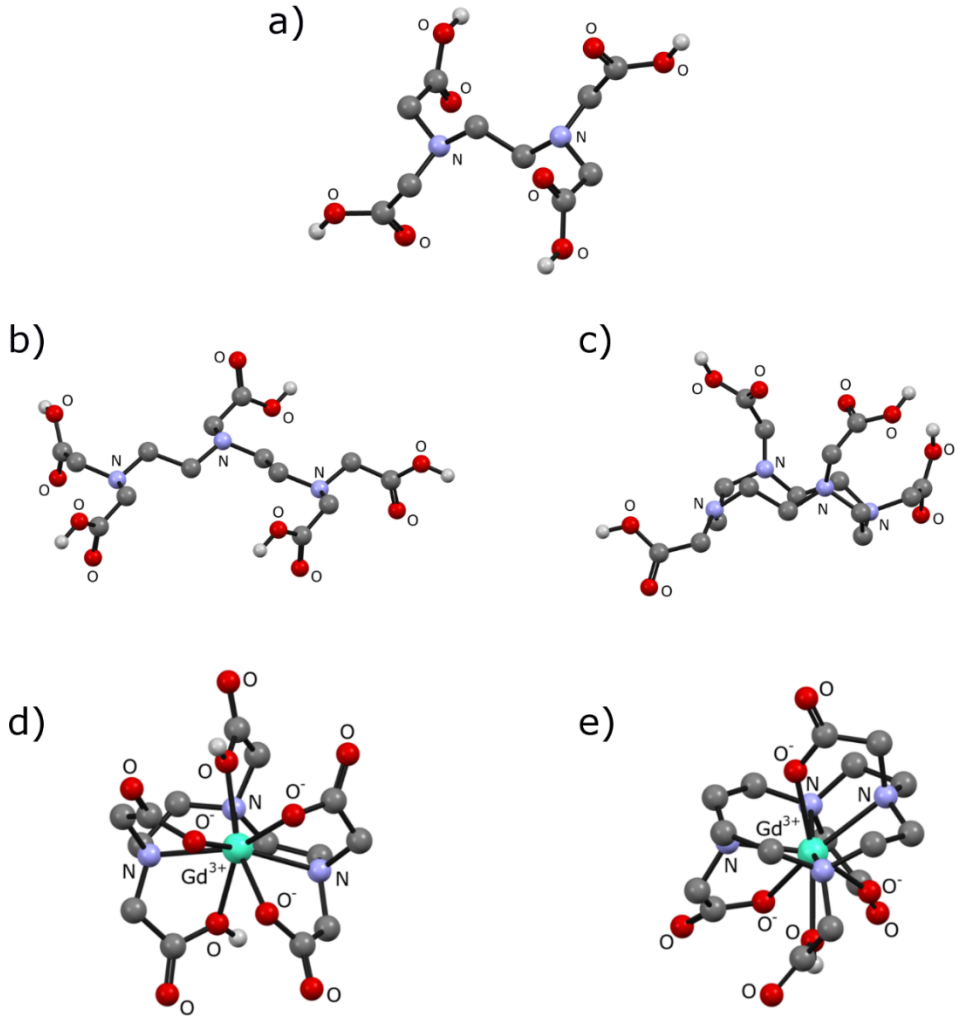

Figure 3. Schematic representations of ligands (used as synthetic scaffolds for *in vivo* imaging) and small-molecule contrast agents in clinical use: (a) EDTA – included to show the similarity between chelates, b) DTPA, c) DOTA, d) Gd-DTPA complex, and e) Gd-DOTA.

Another alternative to Gadolinium(III) is the use of superparamagnetic iron oxide nanoparticles (SPIONs) which are considered to be non-toxic as they are eventually transported out of the body *via* heamoglobin in the blood. These nanoparticles

are the most widely investigated T_2 MRI contrast agents and the most advanced frontier in terms of clinical applications for MNPs. SPIONs exhibit strong T_1 and T_2 relaxation characteristics due to their strong and varying magnetic fields. The relaxation processes observed by SPIONs are explained by classical outer sphere relaxation theory with respect to water diffusion within the particle magnetisation field.[43] The significance of orbital shrinking relaxation (OSR) theory is that the relaxation ration (r_2:r_1) increases with particle size, and SPIONs were initially developed as T_2 agents. However, a new generation of SPIONs have been reported with good r_1 enhancing properties.[44] The magnetic properties of metal-based core-shell nanoparticles enable them to be detected by MRI scanners: These use an intense magnetic field and different radio frequencies to systematically alter the alignment of the resulting magnetisation of atoms in the human body. It is the magnetic field produced by nuclei spin that is detected by the scanner, and it is the magnetic field gradients that cause nuclei in different areas of the body to rotate at different speeds – making up a complete 3D image. However, achieving high spatial resolution with high contrast using MRI of superparamagnetic nanoparticles remains challenging, especially in regions with natural magnetic susceptibility gradients. An alternative approach is direct MRI of hyperpolarized materials with little or no background signal. Hyperpolarised noble gases[45,46] and ^{13}C-enhanced biomolecules[47] have demonstrated impressive image contrast, but are limited by short *in vivo* enhancement times (~ 10 s for noble gases, ~ 30 s for ^{13}C biomolecules[47]). Recent work with nanoparticles have shown that it is possible to improve *in vivo* enhancement time, in particular working with silicon nanoparticles.[48]

Nuclear magnetic resonance (NMR) in silicon has been widely investigated for half a century, and with renewed interest recently in the context of quantum computation.[49] It is known that bulk silicon can exhibit multi-hour nuclear spin relaxation (T_1) times at room temperature and can be hyperpolarised *via* dynamic nuclear polarisation (DNP).[50] The low natural abundance of spin-1/2 ^{29}Si nuclei (4.7%) embedded in a lattice of zero-spin ^{28}Si nuclei isolates the active nuclear spins from one another and from the environment, leading not only to long T_1 times, but also decoherence (T_2) times of up to tens of seconds. Moreover, the weak dipole-dipole coupling of the sparse ^{29}Si atoms, together with the isotropic crystal structure and the absence of nuclear electric quadrupole moment conspire to keep any induced nuclear polarisation aligned with even very weak external fields as the nanoparticle tumbles in space, which occurs, for instance, in fluid suspensions. In this sense, Si nanoparticles have two critical properties for their use as targetable hyperpolarized MRI imaging agents. Firstly, Si nanoparticles retain long T_1 times at room temperature into the submicron regime. Secondly, we demonstrate that long-T_1 Si nanoparticles can be surface functionalised by methods similar to those used to prepare other targeted nanoparticle systems.[51,52]

There are two mechanisms in which the nanomaterial can be directed at the tumour – active and passive targeting.[53] Passive targeting relies on the basis of EPR (enhanced permeability and retention) effect, which allows a nanoparticle to enter the tumour tissue *via* permeable vasculature caused by rapid angiogenesis – common in all cancerous tumours.[53] The retention effect can be enhanced by coating the nanomaterial in poly(ethylene glycol), which prevents the adsorption of blood serum proteins, which can lead to removal of the material, thus extending circulation time and increasing the chance of permeation directly into the cancerous tissue. The active mechanism takes advantage of gene overexpression of a tumour cell. Attaching a ligand corresponding to a receptor on the tumour cell further increases the retention effect, relative to the passive approach.[54] The difficulty is in competing with blood clearance systems, with each mechanism in contention with the other. However, a recent paper by Von Maltzahn *et al*. proposes a more sophisticated way of improving tumour targeting based systems. This involves a two-part system consisting of 'signalling' and 'receiving', specialised nanoparticles that communicate with each other to greatly enhance the drug payload at the diseased site (Figure 4).[55] Metal-based nano-cores are of particular interest due to their diagnostic-therapeutic multimodality and inherent magnetic properties when compared with other nanomaterials also undergoing pre-clinical research (e.g. Silicon based cores and allotropes of carbon such as carbon nanotubes, which can also be used in magnetic resonance imaging *in vivo*).

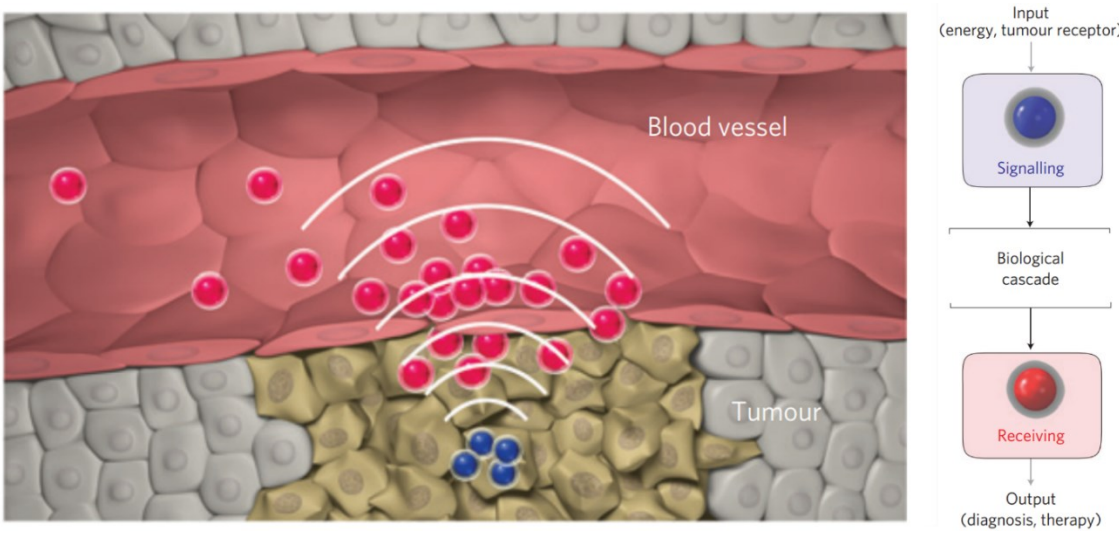

Figure 4. A schematic diagram of a signaling cascade adapated from the work of Von Maltzahn *et al*.[55] This 'smart' method involves signalling units (such as gold nanorods) which communicate with receiving species in circulation (such as liposomes). These species, whether diagnostic or therapeutic can actively seek out the tumour site.

2.1 Noble metal nanoparticles as probes for bioimaging

Metallic nanoparticles (NPs) have been a major focus on research and progress in the last decade due to their unique chemical and optical properties.[56,57] Indeed, a simple change in size can alter the optical properties of the metallic particles due to a phenomenon known as surface plasmon absorption (SPA).

As size and shape changes, the energy between valence and conductive bands, known as band gap, also changes. This results in a new characteristic absorption - emission profile, which is size and shape dependent and therefore potentially tuneable for bioimaging applications.[58] Noble metal nanoparticles present reduced cytotoxicity compared to semiconductors nanocrystals containing Cd, Se or Te. Indeed, biosensors based on quantum dots undergo leaching of heavy metals, which may induce apoptosis and cytotoxicity and are thus unsuitable for clinical use. Moreover, metallic NPs have a high surface to volume ratio so that they can be used as a scaffold for further functionalisation.

In recent works, AuNPs have been used in conjunction with other nanomaterials, such as graphene sheets, to create a novel sandwich immunoassay, or with mesoporous silica to create nanostructures possessing both imaging and therapy modalities. This is in part due to the ease in which AuNPs can be functionalised, their high loading capacity and their biocompatibility. One of the most common small molecule imaging agents, tri-iodobenzene possesses shortcomings such as renal toxicity, short imaging times and poor contrast in larger patients.[59] A 2006 paper published by Hainfeld *et al.*[59] was one of the first to highlight the use of AuNPs as an alternative contrast agent for X-ray imaging. AuNPs with an average diameter of 1.9 nm were injected intravenously into mice and their distribution measured by atomic absorption. It was found that the images produced possessed high clarity and spatial resolution with clear marking of blood vessels less than 100 μm, whilst there were low levels of liver and spleen retention.

Further research by Popovtzer *et al.*[60] also showed that AuNPs are useful in imaging using computed tomography (CT). Different tissues in the body possess different degrees of X-ray attenuation. Elements with a higher atomic number and electron density have a higher attenuation constant. Gold has an atomic number of 79 and an electron density of 19.32 g cm^{-3}, which is higher than that of the currently used iodine (Z=53, density 4.933 g cm^{-3}).[60] By functionalising the surface of AuNPs with a prostate-specific membrane antigen (PSMA), Jon *et al.* established a targeted molecular CT imaging system of relevance to early prostate cancer diagnosis. In a recent example, a PSMA aptamer acted as a targeting molecule for chemotherapeutic intercalator, doxorubicin (Dox), which resulted in a 1:1 PSMA-Dox complex anchored to gold surfaces (Figure 5).[61] Work carried out by Kim *et al.* demonstrated the possibility of functionalising AuNPs with small peptide molecules, capable of binding to PMSA. It was found that the aptamer-AuNPs complexes showed a CT intensity four times greater for targeted prostate cancer cells than for non-targeted cells. After loading the chemotherapeutic drug doxorubicin, the nanoparticles were found to be more potent nanotherapeutics agents against the targeted prostate cancers cells with respect to non-targeted cells.

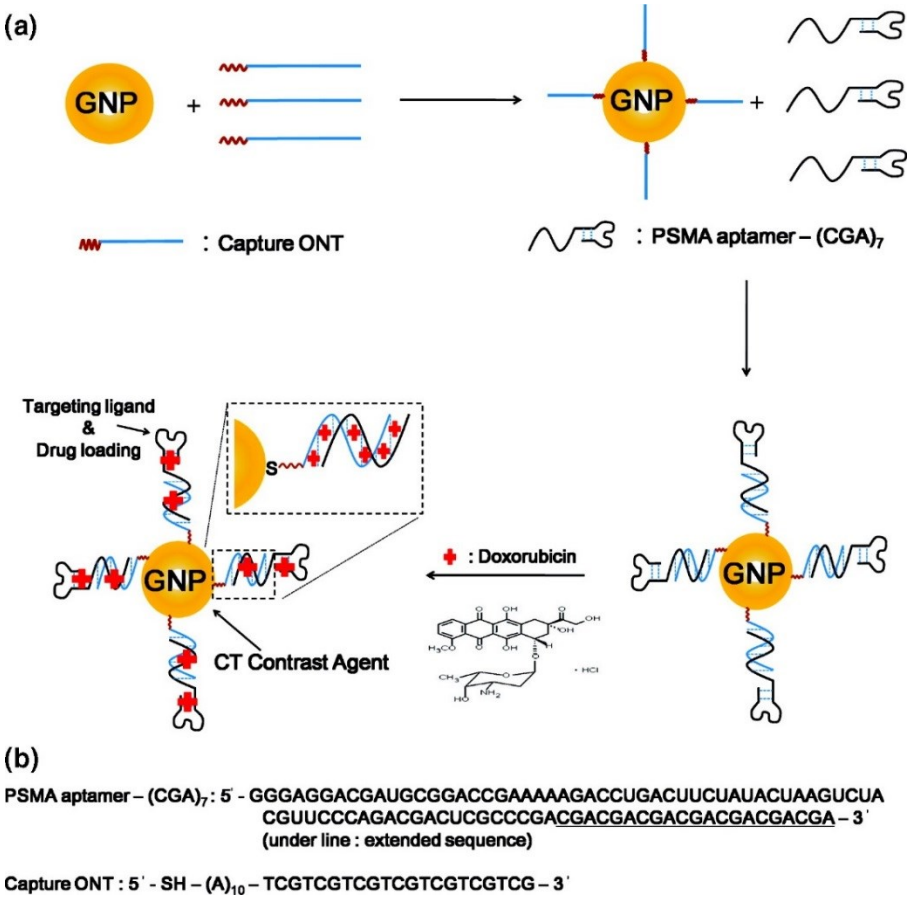

Figure 5. Schematic illustration of a method for preparing Dox-loaded PSMA-AuNPs complex (adapted from Reference 61)

Resonant electron oscillations on the surface of noble metal nanoparticles (such as gold, silver and copper) lead to surface plasmon resonance which enhances light absorption and Raleigh scattering of light which is highly useful for Raman imaging.[62] Oyelere *et al.* focused on changing the shape of AuNPs from spherical to rod-like in order to increase and tune the SPA absorption and scattering from the visible to the near-infrared region of the spectrum.

Lu *et al.*[63] synthesised gold 'nano-popcorn' structures, functionalised with RNA aptamers through amino links and anti-PSMA antibodies and then incubated these with cancerous and non-cancerous cells. The structure denoted 'popcorn' consists of a central AuNP, which acts as an electron reservoir, whilst the surrounding AuNPs are able to focus the Raman field at their apexes (Figure 6).[63] It was found that the novel-shaped nanoparticles would aggregate at cancerous cells forming "hot spots" which can be registered by surface-enhanced Raman scattering (SERS) imaging. Furthermore, it was found that there was a Raman signal enhancement of 2.5×10^9 and that prostate cancer cells could be detected at a 50-cell limit. The nano-popcorn also was capable of differentiating between normal and cancerous cells and there was a linear relationship between the percentage of cancer cell death and the SERS intensity change. This has promising applications in combined imaging and sensing of cancer cells for early diagnosis in a non-invasive way.

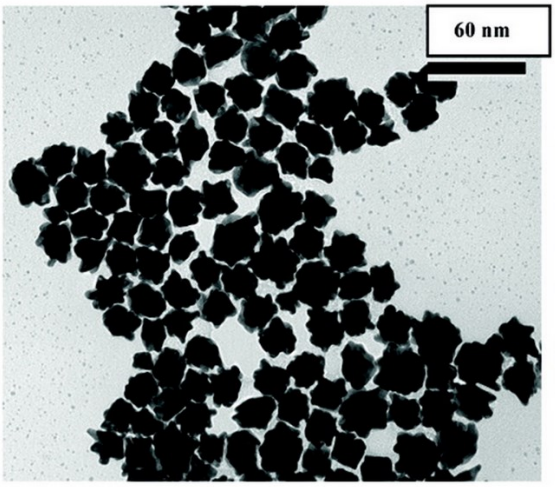

Figure 6. A TEM image of anti-PSMA antibody conjugated gold nanopopcorn (adapted from Reference 63)

A recent study by Kasten *et al.*[64] built upon this research and extended it by looking at the possibility of binding PSA inhibitors to AuNPs. Inductively coupled plasma atomic absorption spectroscopy was used to evaluate cancerous tissue samples which had been incubated with the inhibitor-functionalised nanoparticles and found good cell uptake in prostate cancer cells.

Nienhaus *et al.*[65] have reported a facile strategy of synthesising gold nanoclusters (NCs) functionalised with dihydrolipoic acid (DHLA) (Figure 7). This provides a valuable methodology of anchoring and immobilising biological molecules *via* covalent linkage or electrostatic interactions. Moreover, in a recent example, NCs exhibited near infrared luminescence and a fluorescence lifetime two orders of magnitude longer than the lifetime of cellular autofluorescence. Thus, this work showed that DHLA-AuNCs are indeed valuable fluorescence markers for fluorescence lifetime imaging.[66]

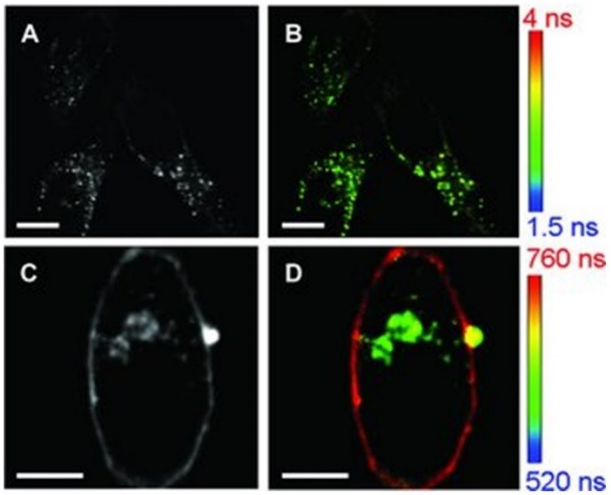

Figure 7. Intensity (A, C) and FLIM (C, D) images of cells (A, B) and cell incubated with 100 μg/mL of DHLA-AuNPs. Scale bar: 10 μm (reproduced from Reference 65)

In 2005, Roux *et al.*[66] reported their explorations into use of AuNPs as a carrier for gadolinium chelates such as DTPA. To achieve this, the authors proposed an *in situ* reaction involving a gold salt and a dithiolated derivative of DTPA. X-ray photoelectron spectroscopy (XPS) revealed the presence of a shell containing about 150 ligands on 2–2.5 nm sized AuNPs. These particles exhibit a high relaxivity ($r_1 = 585\ mm^{-1}\ s^{-1}$, compared to $3.0\ mm^{-1}\ s^{-1}$ for DTPA:Gd), rendering them very attractive as contrast agents for MRI applications *in vivo*.

Recently, Gonnlaugson *et al.*[67] further developed the concept of anchoring luminescent lanthanide species onto nanoparticles. They designed novel hybrid nanomaterials as MRI and luminescent probes for sensing and imaging applications. In this work, the authors reported that such luminescent lanthanide complexes can also be conjugated to various types of NPs, non-metallic as well as metallic, e.g. polystyrene, silica, silver, magnetite and gold-magnetite hybrids.

In 2014, Ricciardi *et al.* reported a synthetic strategy for obtaining a multifunctional material for both imaging and tumour therapy. Octahedral Ru(II) and Ir(III) bipyridine complexes onto Au@SiO_2 core-shell nanoparticles. Transition metal doped Au@SiO_2 nanoparticles generate singlet oxygen against tumor cells and simultaneously retain luminescent properties of the metal complex (Figure 8).[68] Gold nanoparticles has been recently used for the colorimetric detection of Cu^{2+} ions. Fu and collaborators reported the assembling of phosphatidylserine (DOPS) on the AuNPs. In a recent example NPs were shown to selectively bind Cu^{2+} leading to an aggregation of nanoparticles and consequently change color from a wine red to blue (Figure 9).[69]

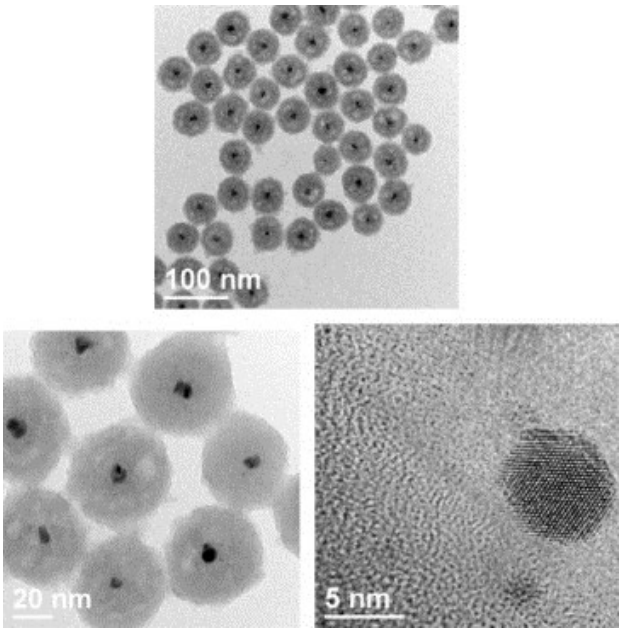

Figure 8. TEM imaging of Au@SiO_2 core-shell nanoparticles doped with a Ru(II) bipyridine complex (reproduced from reference 68).

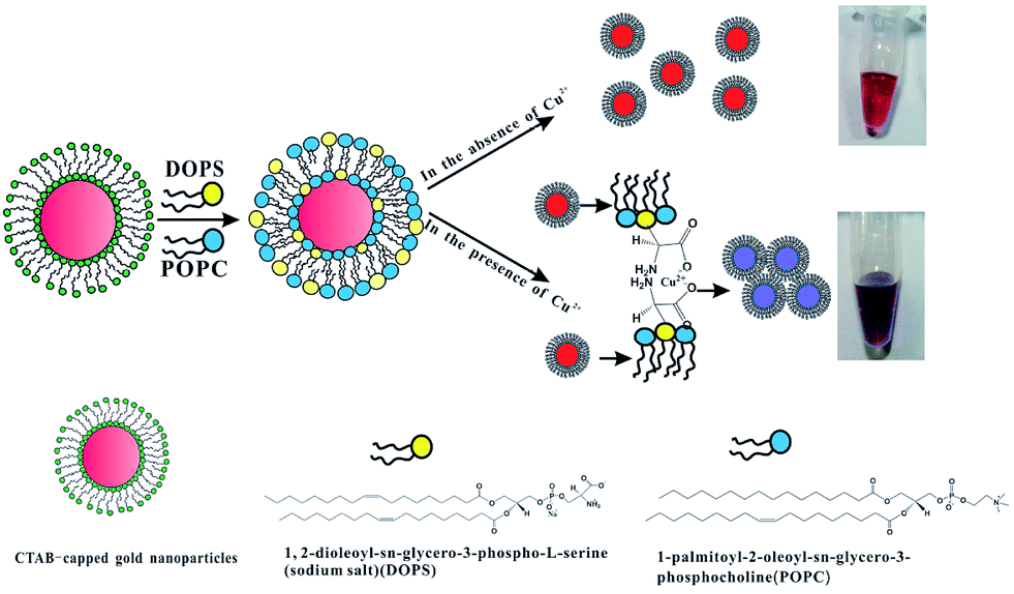

Figure 9. Schematic illustration of the detection principle of DOPS functionalised AuNPs (reproduced from reference 69)

2.2. Metal alloys and metal oxide nanoparticles for bioimaging

A recent popular design for nanomedicines focuses on the use of metal alloys in addition to metal oxides, including transition metal elements with unpaired electrons (paramagnetic), such as Cr, Mn, Fe, Ni, Co or Gd, and the lanthanide series.[70] Won Seok Seo and colleagues used FeCo/graphitic shell nanocrystals as an advanced MRI and near infrared agent, discovering its superior magnetism. FeCo and Fe are among the strongest known magnetic materials, but are susceptible to oxidation and corrosion which is particularly problematic for any biomedical application. Without a suitable coating, use in a biological context is in vain. Metal oxides coined super paramagnetic iron oxides SPIONs and ultra-small super paramagnetic iron oxides (USPIONs)[71] have been thoroughly researched as they generally have an innocuous toxicity profile and have acceptable magnetisations. Another advantage of iron-oxides is that iron is already a main component in red blood cells and can be added to the body's iron store (mainly in the liver) after particle degradation. For these reason, Fe_3O_4 has been used as coating material for platinum-iron alloy surfaces.

Manganese is also another trace element found in the body, however, its potential for cumulative harm is a lot higher than iron. The other transition elements previously stated are highly toxic *in vivo*. They would require a chelate system or polymer based coating to prevent direct contact with the biological environment,[71] and as of yet have not been thoroughly researched due to their inherent toxicity in living systems. Iron-alloy based cores have shown successful results and some have already reached the clinical phase. The latter study concentrates on cytotoxicity, however imaging was predominantly done using a superconducting quantum interference device, exploiting quantum superposition, therefore directly monitoring the FePt nanocrystal. It has been shown in a recent review by Sun *et al.* that, within the structure of metal alloys such as FePt, the interaction between the two transition metal elements gives rise to greater chemical stability than the single element analogue.[72]

The proposed mechanism for the anticancer properties of $FePt/CoS_2$ core shell nanoparticles is based on the hypothesis that the iron centre in the FePt unit is susceptible to be oxidised under an acidic environment *in vitro*, which leads to the release of Pt^{2+} ions. In turn these may penetrate the nucleus of the cell and/or mitochondria thus inducing apoptosis through DNA binding (Figure 12). Other systems such that FePt–CdS, FePt–CdSe, FePt–Au and FePt–ZnS, have also been exploited due to their dual optical and magnetic attributes.[71] However, it was found that the FePt magnetisation is low (i.e ~10 emu g^{-1} at 0.5 kOe applied field) relative to FeCo, and thus too small to be applied for MRI research.

Chih-Wei Lai and colleagues synthesised an $FePt/Fe_3O_4$(oxide alloy hybrid) core-shell nanomaterial under solvothermal conditions.[71] They found a 6-fold enhancement in magnetisation in comparison to that of FePt(Fe_3O_4 Ms ≈ 14.0 emu g^{-1}).[73] Additionally, they discovered two unique properties of these systems: high dispersibility as well as a particularly labile nature i.e phase transfer to water-soluble $FePt/Fe_3O_4$ coated with 2,3-dimercaptosuccinic acid (DMSA). This discovery will no doubt increase future interest in alloy/oxide coupling for biomedical applications (Figure 10).

Gold coated iron-oxide Fe_3O_4 were also reported, having saturation magnetisation values for the uncoated and gold-coated Fe_3O_4 nanoparticles of 30 and 4.5 emu g^{-1} respectively.[74] The use of gold/iron hybrid NPs is advantageous as gold iron nanoparticles can be reliably modified through thiol chemistry which in turn may link these nanoparticles with diverse biomolecules of relevance to cancer diagnosis. However, such Au-coated magnetic nanoparticles have found rather rare applications: it has been suggested that this may be due to difficulties in synthesis as the procedure is very time consuming, heating to high temperatures is problematic, and further chemical modifications need to be considered to prevent particle aggregation.

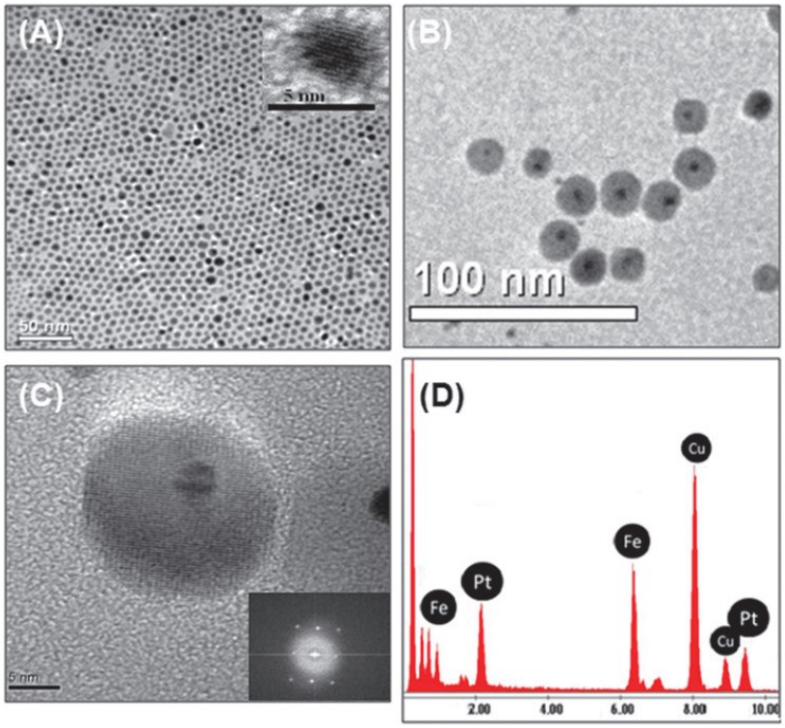

Figure 10. (A) TEM micrographs of FePt MNPs and (B) 15.5 nm $FePt/Fe_3O_4$MNPs. (C) HRTEM image of $FePt/Fe_3O_4$ and the correspondingelectron diffraction Fourier transform image (inset). (D) EDX spectrum of $FePt/Fe_3O_4$ (reproduced from Reference 71)

Metal oxide nanoparticles have been extensively used in healthcare and manufacture products.[75] Metal oxide-based nanocrystals were the most investigated nano-dimensional contrast agents in MRI. Magnetic contrast agents in MRI are used to modify the relaxation time of protons in the tissues where they accumulate, which causes changes of MR signal intensity and consequently the imaging contrast. The image contrast is achieved by the differences in the NMR signal intensity in different areas within the object. The NMR signal intensity largely depends on the nuclear density (proton spins), the relaxation times (T_1, T_2, and T_{2*}), and the magnetic environment of the tissues. By decreasing T_1 and T_2 of normal tissue surrounding a

tumour, the contrast between healthy and tumour tissues is enhanced. Among those contrast agents are (Fe_3O_4) and magnemite (γ-Fe_2O_3), both having spinel structures and belonging to the ferrite metal oxide family. They are biocompatible and generally non-toxic in living systems on a concentration scale suitable for most diagnostic applications, while their strong magnetism still allows for successful remote manipulation.

Recently, super paramagnetic iron oxide particles were used as cores in a novel dual modality PET/MRI probe.[76] The work of Bao and co-workers is an example of the versatility of the iron oxide surface with regards to functionalisation. The authors described a synthetic protocol leading to a PEGylated phospholipid coating of SPION. The chelator 1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetraacetic acid (DOTA) was physically absorbed into the PEGylated micelle to achieve labelling with positron-emitting ^{64}Cu. This dual-imaging PET/MRI nanoparticle produced strong MRI and PET signals and showed stability in serum with respect to decomposition for 24 h.

One particular aspect of these NPs is the evaluation of their fate once these are injected in a biological system (Figure 11). Rees, Llop and co-workers investigate the relationship between NPs size and site of their accumulations *in vivo*. They demonstrated that direct irradiation of Al_2O_3 NPs with 16 MeV protons generates the positron emitter ^{13}N capable of performing biodistribution studies up to 68 minutes post intravenous administration.[75]

Chih-Wei Lai and colleagues synthesised FePt/Fe_3O_4 nanoparticles in a one-pot solovothermal process involving a two-step process.[71] This work reported highly uniform core shell nanoparticles *via* this solvothermal technique which was described as a facile approach. Jinhao Gao and colleagues reported the synthesis of FePt@CoS_2 Yolk-shell nanocrystals,[77] suggested an explanation for their formation (based on the Kirkendall effect), and proposed a mechanism for the nanoparticles entering and killing HeLa cells (Figure 12).

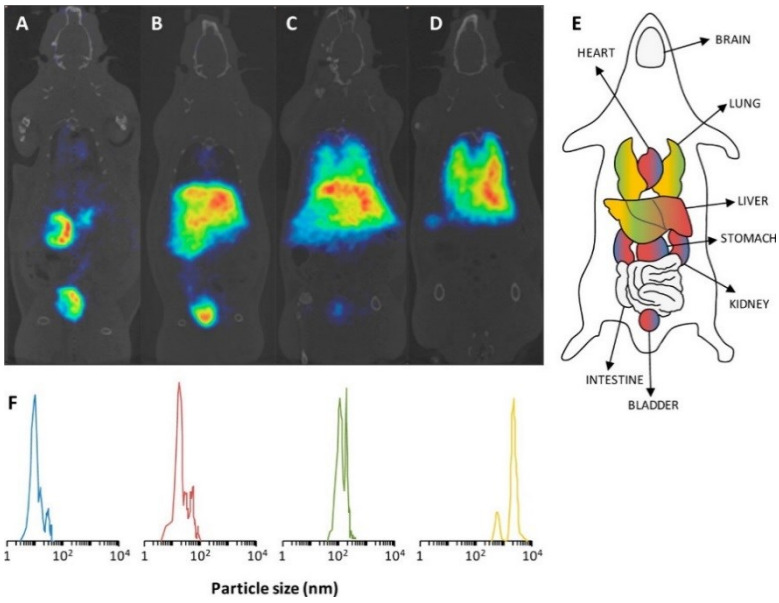

Figure 11. (A-D) PET images of ^{13}N-labeled Al_2O_3 NPs signal at t = 60 min: NS 10 nm NPs (A), NS 40 nm NPs (B), NS 150 nm NPs (C), and NS 10 µm NPs (D). CT images were adjusted along the y-axis for an appropriate fitting with the tracer distribution image. (E) Organ accumulation as a function of particle size, according to the colour code depicted in F. (F) Particle size distribution as determined by TEM (NS 10 nm, NS 40 nm, NS 150 nm) or DLS (NS 10 µm); grey color in E indicates low accumulation irrespective of particle size (reproduced from Reference 75).

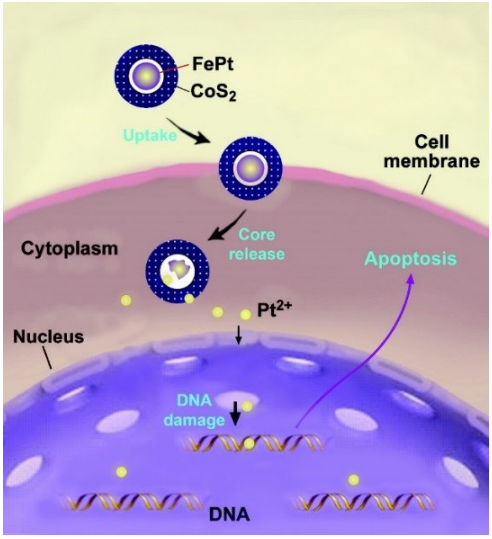

Figure 12. FePt nanoparticles are first oxidised to form Fe(III) and Pt(II). The Pt(II) ion then passes through phospholipid bilayer of the cell. This results in apoptosis of the cell. In this process, the cell shrinks and pulls away from neighbouring cells. Blebs appear on the surface of the cell and chromatin condenses on the surface of the nucleus. The entire cell then breaks up into fragments, which are to be ingested by the surrounding cells (reproduced from Reference 77)

2.3. Super-resolution methods in optical microscopy using metal nanoparticles *in vitro*

The concept of super-resolution microscopy and its applications in optical imaging in living systems was introduced for the first time in 1994[78] and since then it has become a very attractive field of research for several research groups worldwide, leading to the award of a Nobel Prize for Chemistry in 2014 to Eric Betzig, Stefan W. Hell and William E. Moerner, for their discovery of 'Super-Resolved Fluorescence Microscopy'.[79-81]

Recently, photoactivation localisation microscopy (PALM) techniques have been used to study surface-enhanced fluorescence (SEF) on metal nanostructure. Silver nano-wires and gold nanoparticles were synthesised and functionalised with biotin-Dronpa complexes. Uji-i and collaborators showed that localisation of individual molecules can be successfully combined with the enhancement of fluorescence *via* metal nanostructures (Figure 13).[82]

In 2014, tertra-spiropyrans titanate [$Ti(SP)_4$] was used as precursor for biodegradable SP-containing biodegradable poly(ε-caprolactone) (PCL) nanoparticles (SP-PCL). Liu, Zhu, Huang *et al*. reported that nanoparticles such as SP-PCL are visible with sub-50 nm resolution in living cells utilising localisation-based super-resolution microscopy, which is much higher

than that obtained using conventional fluorescence imaging (Figure 14).[81] This sets up the hope that multimodality imaging techniques (optical imaging and PET/SPECT agents) and MRI are close to becoming a real possibility towards more accurate diagnosis from cellular to tissue to organ level imaging.

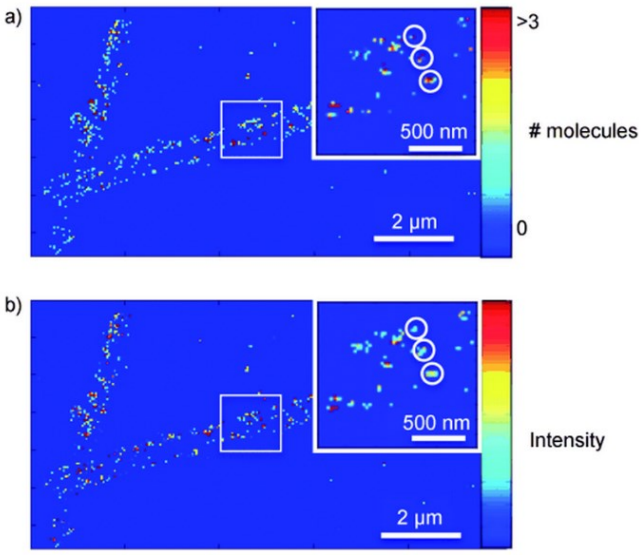

Figure 13. Molecular density maps (with number of fluorescence events per 48 x 48 nm^2) (a) and fluorescence intensity map (maximum intensity detected per 48 x 48 nm^2) (b). The insets are magnified images of the white squares along the wire shown with a bin size of 24 x 24 nm^2 and fluorescence intensity map (maximum intensity detected per 48 x 48 nm^2). (reproduced from Reference 82)

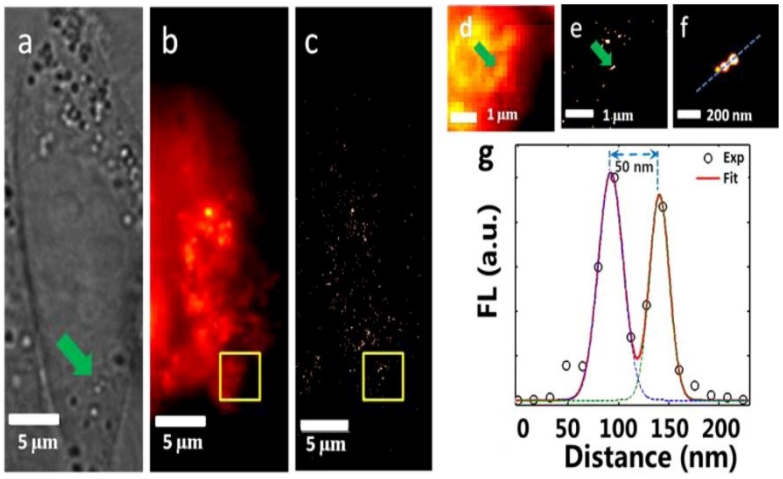

Figure 14. Super-resolution imaging of SP-PCL nanoparticles in live cells. (a) A bright-field image displaying the position of SP-PCL nanoparticles in the cell. (b) A conventional fluorescent image displaying the distribution of SP-PCL in live cells. (c) A super-resolution fluorescence imaging for (b). (d) [72]A magnified view of the boxed region in (b). (e) A magnified super-resolution image of the marked region in (c), corresponding to (d). (f) A magnified view of the marked region in (e). (g) A fluorescence cross-sectional profile of two vicinal SP-PCL nanoparticles along the dashed lines in (f). (reproduced from Reference 81)

3. The immune system and clearance routes for metallic nanoparticles

Before designing contrast agents and their nanodiagnostic counterparts for cancer diagnostic applications, their potential interactions with biological media *in vivo* must be considered. A major difficulty in achieving successful treatment is the human body's immune system. Once a substance or foreign body is taken up within a living organism, it will eventually be filtered out from the blood, e.g *via* the lymphatic system, the renal system and cell uptake *in vivo*. Therefore it is important that the nanomaterial can be engineered to remain in the body long enough, while ultimately maintaining its biocompatibility at the cellular level and crucially, to be broken down for eventual removal, avoiding cumulative toxicity.

The mononuclear phagocyte system (MPS) (found in the liver, spleen, and lymph nodes) is the human body's main way of removing harmful or alien material from circulation.[38] An intimately linked family of blood proteins becomes activated, initiating a cascade, which marks them up for elimination by cells in the MPS. This process is referred to as opsonisation – "the non-specific fouling of plasma protein on the surface of MNPs, and subsequent uptake by reticulo-endothelial system (RES)".[43] It is therefore desirable for nanoparticles to prevent this process, prolonging its circulation time *in vivo*. Meanwhile, it was reported that charged and hydrophobic particles are most prone to opsonisation, while neutral and hydrophilic surfaces are of least suspicion of the body's defences.[77]

Another key consideration in synthetic design is particle size. Larger particles have more of an effect on the body's sensory elements and are more likely to initiate an immune response. However, if the nanoparticles are too small, they will be quickly filtered by the renal system (typically less than a hydrodynamic size of 5.5 nm), and so a compromise must be struck with regards to particle size.[83] Another potential and dangerous outcome is that the body simply cannot remove the nanomaterial from the body leading to cumulative toxicity, making biocompatibility a major consideration during the design of any nanomedicine.[84]

4. Conclusion and outlook

We included here a survey of those metallic nanoparticles which have been a matter of intense exploration within the last decade due to their potential to change the face of the medical world through their role as 'nanotheranostics'. Some of the current probing techniques for multimodal imaging potential with a main focus on metallic nanoparticles, alloys and their

derivatives were highlighted drawing on the published synthetic protocols for core-shell nanoparticles. Such systems undoubtedly have an important role to play in the future of nanomedicines design particularly as they can be tailored to act as synthetic platforms towards for cancer diagnosis using modern molecular imaging techniques (CT, PET, SPECT and MRI) and treatment. A range of metallic nanoparticles and their potential applications as bio-nanomaterials as cancer nanotheranostics were highlighted. Targeting ligands, fluorophores, genes, and chemotherapeutic agents can be added to the surface of the metallic nanoparticle, the further increasing the functionality for this recent technology, at the forefront of pre-clinical investigations. The metal-based colloids, alloys and metallic nanoparticles discussed in this review show promise as cancer nanodiagnostics as evident from the recent publications landscape: we included here only those metallic nanoparticles which are already having established applications *in vivo* as well as some of the new metallic nanoparticles with potential as cancer nanodiagnostics but which are currently awaiting *in vitro* and *in vivo* evaluation.

Critically, the future of nanomedicine as a whole, although bright, requires some further detailed investigations in order to overcome some of the main synthetic limitations hindering its progression towards becoming a useful tool for cancer diagnosis. These include the current need for rigorous investigation into the short-term and long-term effects *in vivo* of such materials and a complete mechanistic understanding on a molecular scale to predict further hazards to living systems, as well as the environment. Currently, there remains the case that a comprehensive risk profile of nanotechnology has not yet been agreed, however, there have been considerable efforts attempting to address the toxicology and safety of nanomaterials and some were highlighted in recent reviews.[85] The financial and ethical associations with nanomedicine need to continue to be closely considered and monitored, such that the impact of this research on the current diagnosis and treatment of cancer is realised.[86]

The benefits of this field are clear, since targeting nanomedicines as drugs can be completely localised to the site of disease, requiring lower doses overall, and avoiding the unpleasant side effects that are associated with current methods of cancer treatment. The promising application of magnetic nanoparticles in molecular imaging therefore provides great hope for the diagnostic era of nanomedicine with clear benefits towards early diagnosis and treatment of cancer patients.

Notes and References

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